



Toxicity of Terpenes Secreted by the Predator *Xylocoris flavipes* (Reuter) to *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.)

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Abstract—Four terpene alcohols, linalool, geraniol, α -terpineol, and nerol, which are compounds produced by *Xylocoris flavipes* (Reuter), were tested for toxicity against adults of *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.) using a Petri dish assay. Dose-response studies were conducted for each compound singly and in a combination that mimicked the concentrations of these volatiles in exocrine secretions of *X. flavipes*. Linalool and α -terpineol were toxic to *T. castaneum* in a dose-dependent fashion, but geraniol and nerol were not toxic during the 24 h bioassay. The mixture of the four compounds was several times less toxic than linalool and α -terpineol for *T. castaneum*, even when exposed to large amounts. All four terpene alcohols and the mixture were toxic to *O. surinamensis*, with α -terpineol proving most toxic and linalool the least toxic. Toxic effects of linalool and α -terpineol against *O. surinamensis* occurred within very narrow ranges, suggesting the possibility of a threshold concentration. Variation in toxicity among similar compounds and between insect species for the same compounds should be examined in studies that assess terpenoids for toxicity against stored-product insects.

Key words—Terpenes, Coleoptera, Tenebrionidae, Cucujidae, natural products.

INTRODUCTION

There is a growing interest in the use of natural products to control insects (Cutler, 1988; Hedin, 1990). In stored products this interest is driven by a need for alternatives to traditional fumigants and residual pesticides that are becoming ineffective due to resistance in insect populations, or are no longer available due to regulatory controls (Civerolo *et al.*, 1993). Terpenes are well known as toxicants and behavior modifying chemicals for many species of insects (e.g. Ryan and Byrne,

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1988). Recent studies have shown that crude plant preparations or extracts can reduce experimental populations of stored-product insects (e.g. Weaver *et al.*, 1992, 1995; Regnault-Roger *et al.*, 1993), and some indicate that certain terpene alcohols of natural origin are responsible for toxicity (Shaaya *et al.*, 1991; Weaver *et al.*, 1991).

We investigated the toxicity of four monoterpene alcohols: linalool (3,7-dimethyl-1,6-octadien-3-ol), α -terpineol ($\alpha,\alpha,4$ -trimethyl-3-cyclohexene-1-methanol), nerol, ((*Z*)-3,7-dimethyl-2,6-octadien-1-ol), and geraniol ((*E*)-3,7-dimethyl-2,6-octadien-1-ol) (Fig. 1) against two species of stored-product beetles. We chose these four compounds because they are the major components emitted by the predatory pirate bug *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) as a presumed defensive secretion (Phillips and Parajulee, unpublished data). The pest species *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae) were selected for this study because these species occur in the same habitat as *X. flavipes*, and past research demonstrated that *X. flavipes* could suppress populations of these pests in biological control experiments (Press *et al.*, 1975; Arbogast, 1976). We hypothesized that, in addition to predation, *X. flavipes* may have a negative impact on pest populations due to secretion of potentially toxic volatiles. Our objective in the present study was to assess toxic activity of linalool, α -terpineol, nerol, and geraniol, singly and in a four-component mixture, against adults of *T. castaneum* and *O. surinamensis* in a series of dose-response experiments.

MATERIALS AND METHODS

Insects

Insects were obtained from cultures in our Madison laboratory that were maintained at 29°C, 60% r.h. and 16:8 (L:D) photoperiod. *T. castaneum* was collected from corn stored on a farm in Waunakee, WI, U.S.A. in September, 1990 and reared on a mixture of whole wheat flour and brewer's yeast (95:5) in the laboratory since that time. *O. surinamensis*, originally obtained from the Stored-Product Insects Research and Development Laboratory, Savannah, GA, U.S.A. had been in culture for over 10 y and was reared on a mixture of rolled oats and brewer's yeast (95:5). Adults for bioassays were sieved from colonies 1 week after emergence and held for a minimum of 1 h before use.

Bioassay

We used a Petri dish bioassay in which the test compound was applied to an absorbent substrate and mortality resulted from fumigant exposure and/or contact toxicity. Terpene alcohols (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) were racemic, at least 95% pure, and dissolved in HPLC-grade N-hexane for testing. We used disposable plastic Petri dishes, 60 × 15 mm, with 55 mm filter paper inserts (Whatman No. 1) fitted to the floor. A 250 μ l volume of hexane solution was applied to the filter paper and allowed to dry for 5 min. Preliminary studies determined that 250 μ l would completely cover the filter paper without runoff, and that all solvent was evaporated in 5 min. An experimental unit consisted of 10 insects placed in a treated Petri dish for 24 h at 22°C and 40% r.h. Mortality was assessed by immobility of the insects and a habitus characteristic of death. Immobile insects were removed from the dish and touched with a probe several times to further assess mortality. Ten replicates of each treatment and the control (250 μ l of hexane only) were deployed. Preliminary studies were conducted with doses of 7000 μ l of each compound in hexane to determine levels of mortality for the two beetle species and project future test

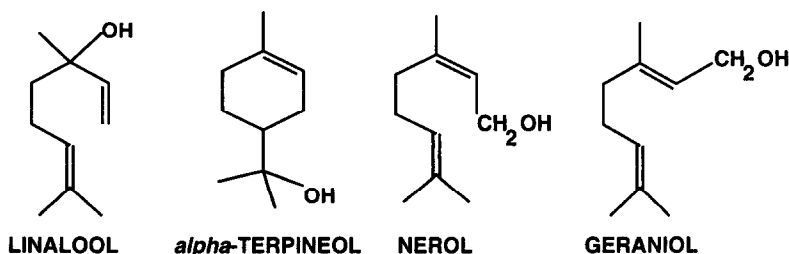


Fig. 1. Molecular structures of four monoterpene alcohols used in this study.

concentrations. All experiments employed at least one low dose that yielded mortality similar to controls and one high dose that typically resulted in 100% mortality; three intermediate doses were evaluated when possible. Doses for *T. castaneum* ranged from 1783 to 23,768 μg , while those for *O. surinamensis* ranged from 112 to 2020 μg . Each terpene alcohol was assessed for toxicity singly, as was a mixture of all four compounds with the following composition: 76.0% linalool, 14.9% α -terpineol, 6.3% geraniol, and 2.8% nerol. The composition of this mixture approximated that quantified in the volatile secretion of *X. flavipes* (Phillips and Parajulee, in preparation). Two compounds, geraniol and nerol, elicited negligible mortality in *T. castaneum* at 24 h, so their effects were assessed by applying 250 mg of undiluted material and monitoring mortality over a 4-day period relative to untreated controls.

Data analysis

Percent mortality in 24 h was computed for each dose applied to the filter paper. Probit analysis for each compound and the mixture was calculated for each species using the PROC PROBIT routine of SAS (SAS Institute, 1989) in which probit-transformed proportion mortality was regressed against \log_{10} dose. Values for LD_{50} , LD_{95} , slope, and y-intercept were generated. LD_{50} values provide a general assessment of toxicity, while LD_{95} values indicate doses required to kill a majority of the test insects. Differences in lethal doses were assessed with the lethal dose ratio test, and differences in slopes and intercepts were assessed with likelihood ratio tests of equality and parallelism of probit lines (Robertson and Preisler, 1992). Dose-response curves were generated as plots of back-transformed probit responses (Throne *et al.*, 1995a) across the range of doses used in the experiments. Exposure time-response data for experiments of nerol and geraniol with *T. castaneum* were also subjected to probit analysis (Throne *et al.*, 1995b).

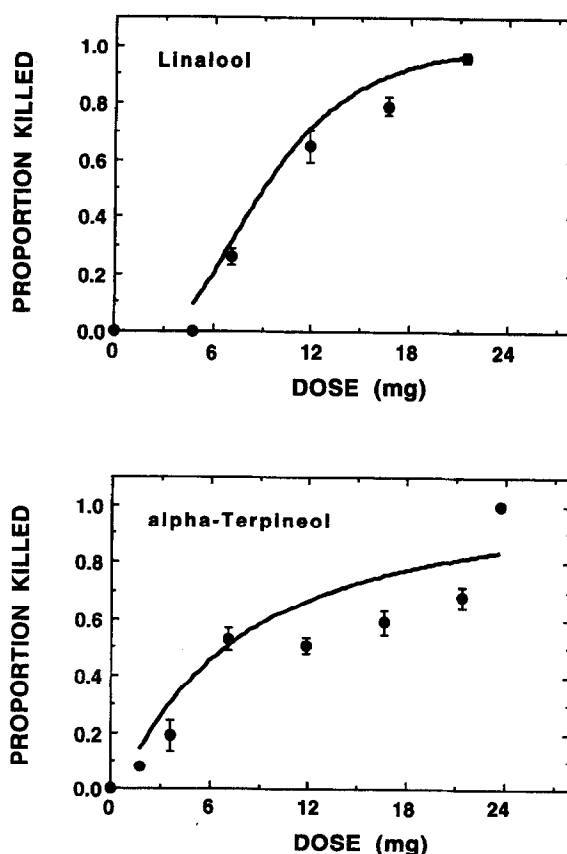


Fig. 2. Mean proportion (\pm SEM) of *T. castaneum* killed following exposure to different doses of linalool (top) and α -terpineol (bottom) in 24 h Petri dish bioassays. Curves are plots of back-transformed probit responses across the range of doses used in the experiments.

Table 1. Toxicity of four terpene alcohols to adults of two species of stored-product insects

Compound and insect species	n	Slope (±SE)	Intercept (±SE)	Goodness-of-fit*		LD ₅₀ (95% CL)† (µg)	LD ₉₅ (95% CL)† (µg)
				χ ²	df		
<i>T. castaneum</i>							
Linalool	600	4.73 ± 1.0	-18.73 ± 4.0	25.07**	3	9042 (4436-13,891)	20,116 (13,250-86,428)
α-Terpineol	1000	1.82 ± 0.4	-7.00 ± 1.7	71.07**	6	6972 (2294-14,654)	55,726 (23,431-786,798)
Mixture‡	700	2.03 ± 0.2	-9.12 ± 1.1	3.56	4	30,271 (25,284-39,487)	194,659 (115,852-461,433)
<i>O. surinamensis</i>							
Linalool	400	87.77 ± 12.4	-287 ± 40.6	1.08	1	1865 (1848-1878)	1947 (1929-1976)
α-Terpineol	500	5.14 ± 1.6	-13.4 ± 4.4	24.94**	2	417§	870§
Geraniol	700	6.06 ± 0.7	-16.6 ± 1.9	7.99**	4	548 (482-618)	1024 (858-1406)
Nerol	690	7.52 ± 0.6	-21.0 ± 1.9	7.73	4	635 (602-670)	1051 (962-1185)
Mixture‡	610	10.9 ± 2.5	-31.8 ± 7.3	14.95**	3	829 (653-967)	1167 (991-2594)

*Goodness-of-fit tests significant at α = 0.05, denoted by **; variances and covariances have been multiplied by the heterogeneity factor, H (SAS Institute, 1989), in computing fiducial limits of lethal doses (H: *T. castaneum*, linalool, 8.3; α-terpineol, 12,411.8; *O. surinamensis*, α-terpineol, 12.4; geraniol, 1.99; mixture, 4.9).

†Probit-transformed mortality data; log₁₀ dose used in the regression model.

‡Mixture of linalool (76.0%), terpeneol (14.9%), geraniol (6.3%), and nerol (2.8%).

§LD value considered provisional; fiducial limits not calculated due to insufficient variation in response.

RESULTS

Both *T. castaneum* and *O. surinamensis* were killed upon exposure to doses of terpene alcohols in these experiments. Linalool and α-terpineol were toxic to *T. castaneum* within the 24 h period, and toxicity increased with dose (Fig. 2, Table 1). Although the LD₅₀ (Table 2) values for linalool and α-terpineol were similar for *T. castaneum*, the LD₉₅ values were significantly different (Table 3) and the probit lines differed in both slope and intercept (Table 4). Nerol and geraniol, however, were not toxic to *T. castaneum* during the 24 h test period. When high doses (250 mg) of these two compounds were applied undiluted to the filter paper, substantial mortality was not observed until 96 h (Fig. 3), while no mortality occurred in the control group during the same period. Mortality of *T. castaneum* at 96 h was probably due to suffocation or asphyxiation rather than typical neurotoxicity. A mixture of all four terpene alcohols was considerably less toxic to *T. castaneum* than either linalool or α-terpineol singly (Fig. 4, Tables 1, 2 and 3).

All four terpene alcohols were toxic to *O. surinamensis* in a 24 h period, and toxicity increased with dose (Fig. 5, Table 1). In contrast to results with *T. castaneum*, geraniol and nerol were toxic to *O. surinamensis* during these 24 h bioassays. The mixture of the four terpene alcohols elicited mortality in *O. surinamensis* similar to that for the four individual compounds (Tables 1, 2, 3, and Fig. 6). Response to doses of nerol and geraniol occurred in a typical stepwise fashion for *O. surinamensis*, but mortality from linalool and α-terpineol occurred within a narrower range of doses. This threshold-type activity indicates that the majority of *O. surinamensis* individuals challenged in the bioassays were similarly sensitive to certain doses of linalool and α-terpineol. Probit lines for response of *O. surinamensis* to the four compounds and the mixture were different,

Table 2. Comparison of lethal doses (LD₅₀) of four terpene alcohols when tested for their toxicity against two species of stored-product insects*

Compound and insect species	Linalool	α-Terpineol	Geraniol	Nerol	Mixture
<i>T. castaneum</i>					
α-Terpineol	1.30 ^{NS} (0.79, 2.15)	—	not tested†	not tested†	—
Mixture‡	0.299** (0.17, 0.51)	0.229** (0.12, 0.46)	not tested†	not tested†	—
<i>O. surinamensis</i>					
α-Terpineol	0.224 ^{NS} (0.001, 47.41)	—	—	—	—
Geraniol	0.294** (0.19, 0.47)	1.311 ^{NS} (0.005, 341.1)	—	—	—
Nerol	0.342** (0.33, 0.36)	1.524 ^{NS} (0.007, 322.7)	0.860 ^{NS} (0.19, 3.86)	—	—
Mixture§	1.689 ^{NS} ‡	0.379 ^{NS} ‡	0.496 ^{NS} ‡	0.577 ^{NS} ‡	—

*Lethal dose ratio tests significant at α = 0.05, indicated by **; NS, not significant (Robertson and Preisler, 1992; PROC PROBIT; SAS Institute, 1989). Confidence limits (95%) given in parentheses.

†Mortality was insufficient for a dose-response analysis at the doses of nerol and geraniol tested with *T. castaneum*, so no comparisons were made.

‡Large confidence limits (≈0 and >10¹⁵)

§Mixture as indicated in Table 1.

Table 3. Comparison of lethal doses (LD₉₅) of four terpene alcohols when tested for their toxicity against two species of stored-product insects*

Compound and insect species	Linalool	α-Terpineol	Geraniol	Nerol	Mixture
<i>T. castaneum</i>					
α-Terpineol	0.361** (0.20, 0.66)	—	not tested†	not tested†	—
Mixture‡	0.103** (0.04, 0.24)	0.286** (0.10, 0.78)	not tested†	not tested†	—
<i>O. surinamensis</i>					
α-Terpineol	0.449 ^{NS} (0.001, 133.3)	—	—	—	—
Geraniol	0.526** (0.50, 0.55)	1.172 ^{NS} (0.003, 430.9)	—	—	—
Nerol	0.542** (0.51, 0.58)	1.207 ^{NS} (0.004, 358.4)	0.971 ^{NS} (0.20, 4.72)	—	—
Mixture§	0.074 ^{NS} ‡	0.033 ^{NS} ‡	0.039 ^{NS} ‡	0.040 ^{NS} ‡	—

*Lethal dose ratio tests significant at α = 0.05, indicated by **; NS, not significant (Robertson and Preisler, 1992; PROC PROBIT; SAS Institute, 1989). Confidence limits (95%) given in parentheses.

†Mortality was insufficient for a dose–response analysis at the doses of nerol and geraniol tested with *T. castaneum*, so no comparisons were made.

‡Large confidence limits (≈0 and >10¹⁸)

§Mixture as indicated in Table 1.

but slopes for many of the pair-wise comparisons were similar (Table 4), indicating a similar response to increasing dose.

DISCUSSION

Although the monoterpene alcohols we studied share the same empirical formula and possess similar structures, they varied in their toxic activities within and between the species of beetles tested. However, insect response is not predictable based on molecular structure of the terpenoid. The acyclic geraniol and nerol are simple structural isomers of each other regarding conformation around a double bond (Fig. 1), and each elicited similar toxicity responses (LD values and probit line equality) for *O. surinamensis*. Conversely, geraniol and nerol were both non-toxic for *T. castaneum* during the 24 h bioassay. α-Terpineol had the lowest LD₅₀ and LD₉₅ values for *O. surinamensis*, and it is a cyclic terpene alcohol. Linalool was least toxic to *O. surinamensis* and it is an acyclic terpene alcohol in which the position of the hydroxyl group and one double bond are different from nerol and geraniol.

The mode of toxicity for monoterpenoids is believed to be via competitive inhibition of acetylcholinesterase (Ryan and Byrne, 1988), thus any terpene alcohol that can effectively compete with acetylcholine at the active site of the enzyme should be able to cause toxicity. The mixture of terpene alcohols we tested was as toxic to *O. surinamensis* as either of the four terpene alcohols

Table 4. Likelihood ratio values for tests of equality and parallelism of probit lines for toxicity of four terpene alcohols assayed against two stored-product insect species*

Compound and insect species	Linalool	α-Terpineol	Geraniol	Nerol	Mixture
<i>T. castaneum</i>					
α-Terpineol	388.4** 629.5	—	not tested†	not tested†	—
Mixture‡	2032.0** 459.0**	1506.7** 9.00**	not tested†	not tested†	—
<i>O. surinamensis</i>					
α-Terpineol	4329.3** 1226.3**	—	—	—	—
Geraniol	4646.5** 0.00 ^{NS}	5.7** 55.9**	—	—	—
Nerol	4537.8** 0.00 ^{NS}	276.7** 0.00 ^{NS}	198.3** 22.0**	—	—
Mixture‡	4085.4** 1609.8**	1191.2** 0.00 ^{NS}	3712.5** 0.00 ^{NS}	534.6** 70.6**	—

*Likelihood ratio tests significant at α = 0.05, indicated by **; NS, not significant (Robertson and Preisler, 1992; PROC PROBIT; SAS Institute, 1989). For each pairwise comparison, the first value given is for equality (no significant difference in slope or intercept) and the second value is for parallelism (no significant difference in slope) of the probit lines.

†Mortality was insufficient for a dose–response analysis at the doses of nerol and geraniol tested with *T. castaneum*, so no comparisons were made.

‡Mixture as indicated in Table 1.

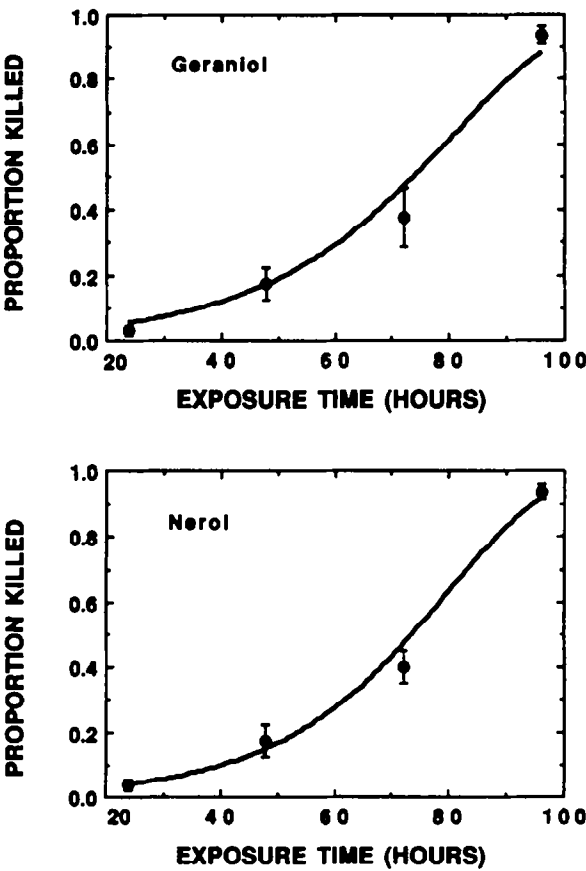


Fig. 3. Mean proportion (\pm SEM) of *T. castaneum* killed following exposure to 250 mg of geraniol (top) and nerol (bottom) in Petri dish bioassays with increased periods of exposure. Curves are plots of back-transformations of Gompertz-transformed mortality regressed against untransformed time (Throne *et al.*, 1995b).

presented separately (Tables 2 and 3). For *T. castaneum*, however, the mixture of terpene alcohols was up to 10 times less toxic than either linalool or α -terpineol singly, which we conclude was due to the admixture of the less toxic nerol and geraniol. The highest dose of the mixture, which killed

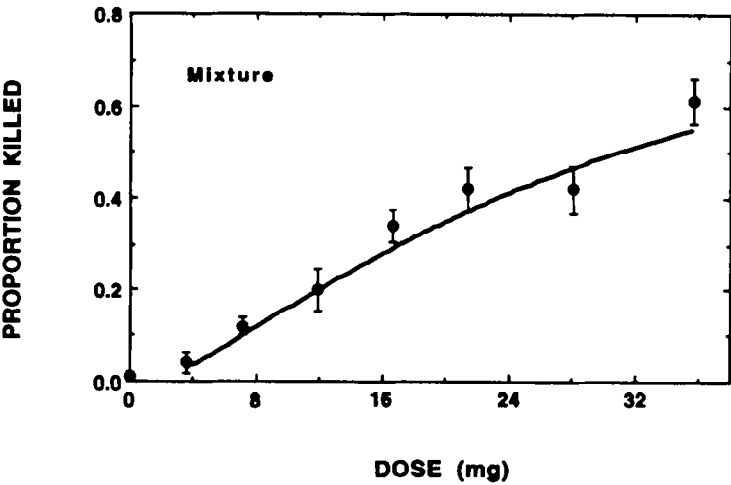


Fig. 4. Mean proportion (\pm SEM) of *T. castaneum* killed following exposure to different doses of a mixture of four monoterpene alcohols in 24 h Petri dish bioassays. Curve is a plot of back-transformed probit responses across the range of doses used in the experiment.

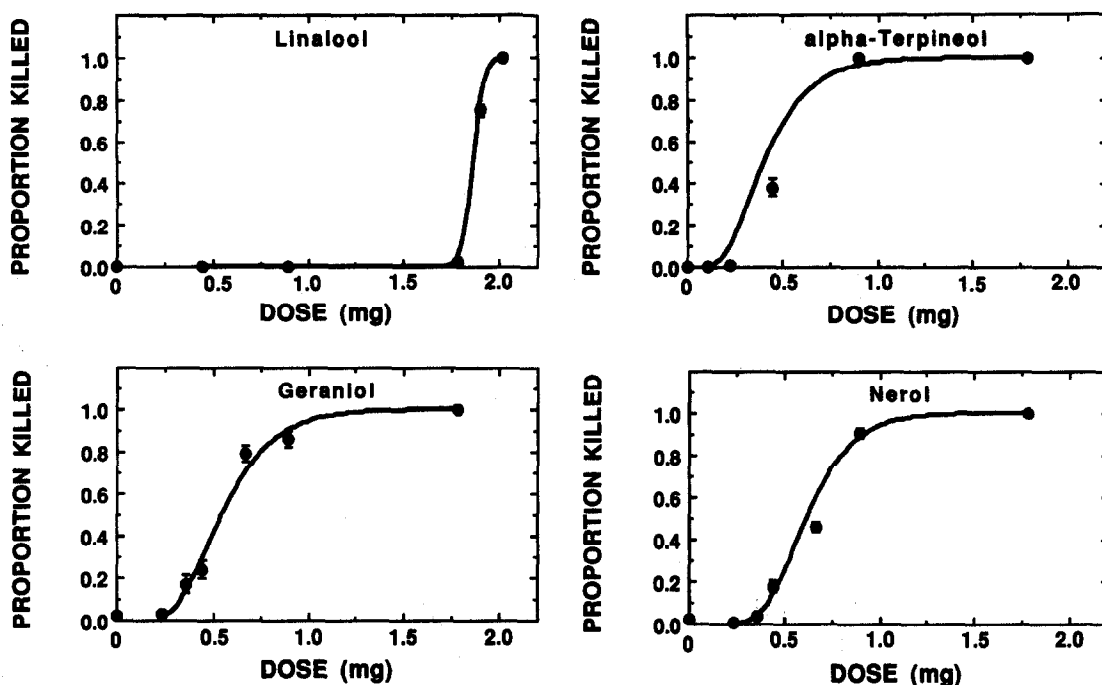


Fig. 5. Mean proportion (\pm SEM) of *O. surinamensis* killed following exposure to different doses of linalool, α -terpineol, nerol and geraniol in 24 h Petri dish bioassays. Curves are plots of back-transformed probit responses across the range of doses used in the experiments.

an average of 63% of test insects per replicate, contained over 27,000 μ g of linalool, a dose greater than one that elicited nearly 100% mortality when linalool was tested separately (Table 1, Fig. 2). Since there were virtually no toxic effects of either nerol or geraniol against *T. castaneum* in 24 h, it is possible that these compounds affected the toxic activities of linalool and α -terpineol either through induced production of metabolic enzymes or by some direct activity on the toxicants at the active site of acetylcholinesterase. Terpenoids are well known inducers of detoxification enzymes in insect guts (Brattsten *et al.*, 1977). If nerol and geraniol are effective metabolic inducers, and if our *T. castaneum* strain was predisposed to metabolic induction by these or similar compounds, then nerol and geraniol present in the mixture may have induced partial detoxification of the more toxic linalool and α -terpineol. Alternatively, nerol and geraniol may somehow inhibit toxicity or competitively displace linalool and α -terpineol at the active site of acetylcholinesterase, but such mechanisms are poorly understood (Ryan and Byrne, 1988). Further study is needed to clarify the bases for toxicity of terpene alcohols in *T. castaneum*.

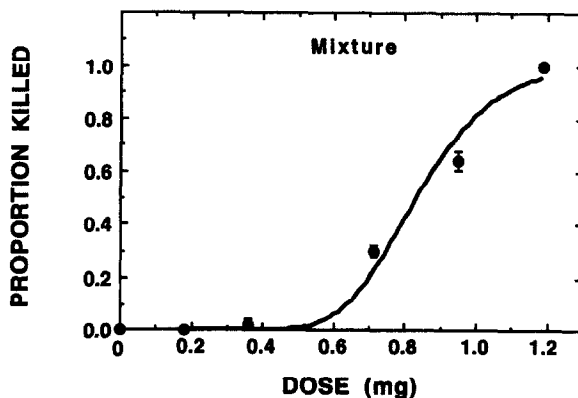


Fig. 6. Mean proportion (\pm SEM) of *O. surinamensis* killed following exposure to different doses of a mixture of four monoterpenic alcohols in 24 h Petri dish bioassays. Curve is a plot of back-transformed probit responses across the range of doses used in the experiment.

Our results corroborate those from other studies that show terpenoids are toxic to stored-product insects (Regnault-Roger *et al.*, 1994; Shaaya *et al.*, 1993), but indicate that structurally similar terpenoids are not similarly toxic to different insect species. We recommend that relative toxicities of different terpene alcohols and mixtures be assessed for various target species in future studies. Recent studies have investigated the toxicity of whole plant extracts, crude essential oils, or simple dried leaves of insecticidal plants for control of storage insects (e.g. Weaver *et al.*, 1992, 1994), and clearly these are typical of initial screening studies. It is possible that, as our work has shown, subsequent assays of individual components rather than natural mixtures are required to give more insight into the potential toxicity of these natural products.

It is unlikely that terpene alcohols produced by *X. flavipes* cause acute toxicity against adults of *T. castaneum* and *O. surinamensis* in the stored-product environment. Minimum doses of terpene alcohols that caused mortality in this study were several orders of magnitude greater than amounts of these compounds produced by small groups (10–20) of bugs in 24 h (Phillips and Parajulee, unpublished data). However, it is possible that immature stages of prey are adversely affected by terpenoid secretions of *X. flavipes*, or that long-term exposure to these compounds in storage systems may have chronic adverse effects on the prey species. We originally hypothesized that a mixture of these four terpene alcohols in the same ratio as that produced by *X. flavipes* would be more toxic than the individual compounds, perhaps due to synergism. This was not true for either prey species, and reduced toxicity was observed for *T. castaneum*. Terpenoid secretions of *X. flavipes* and other Hemiptera are thought to serve a defensive role, particularly against ants (Aldrich, 1988). Since stored-product ecosystems are relatively recent assemblages in evolutionary time (Linsley, 1944), we can not be sure of the context under which *X. flavipes* defensive secretions evolved.

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